

1746-Pos Board B638**Correlative 3-Dimensional Super-Resolution Fluorescence and Tomographic Electron Microscopy of Endocytic Proteins at the Nanometer Scale**Kem A. Sochacki¹, Gleb Shtengel², Harald F. Hess², Justin W. Taraska¹.¹National Heart Lung and Blood Institute, Bethesda, MD, USA, ²HHMI: Janelia Farm, Ashburn, VA, USA.

Three-dimensional super-resolution fluorescence microscopy methods such as interferometric photoactivated localization microscopy (iPALM) can localize individual proteins of interest at high resolution (20 nm in XY and 8 nm in Z). These images, however, lack the structural context that could be provided by other imaging methods including electron microscopy. Here, we develop a method that combines iPALM and 3D tomographic transmission electron microscopy (TEM). First, we use iPALM to localize fluorescently-tagged endocytic proteins on the inner plasma membrane of PC12 cells in three dimensions. Next, platinum replicas of these same membranes are imaged with transmission electron microscopy to create tomograms. Finally, both images are combined to create a map of the nanometer scale location of these proteins within their three dimensional cellular context. This technique has the power to build ultra high resolution 3D topographic maps of molecular structures in the context of their native cellular environment.

1747-Pos Board B639**dSTORM of Synaptic Proteins**Markus Sauer¹, Sebastian van de Linde¹, Thorger Holm¹, Sarah Aufmkolk¹, Nadine Ehmann¹, Robert Blum¹, Dana Bar-On², Amit Alon², Uri Ashery², Dimitrij Ljaschenko¹, Manfred Heckmann¹, Robert Kittel¹.¹University Wuerzburg, Wuerzburg, Germany, ²University Tel Aviv, Tel Aviv, Israel.

Synapses are specialized cell-cell junctions of several hundred nanometers in size that serve as junctions/contact points through which nerve cells send signals to other nerve cells, to muscle cells, or to hormone-secreting cells. Efficient synaptic transmission relies on the precise and specific interaction of synaptic proteins and vesicle fusion and changes in protein dynamics and expression are thought to allow synaptic plasticity. Unfortunately, our understanding of the exact nanoscopic distribution of individual proteins, their absolute numbers and interactions, and changes occurring during learning and memory, and in neurodegenerative disorders is still in its infancy. This is due to difficulties to study sub-synaptic structures e.g. by fluorescence microscopy due to the small size of synapses, which is near the diffraction-limited resolution of light microscopy and the limitation to visualize single synaptic protein localization and dynamics in the synapse.

We demonstrate how super-resolution fluorescence imaging by *direct* stochastic optical reconstruction microscopy (dSTORM) can be used advantageously to study the distribution of synaptic proteins with an optical resolution of 15-20 nm in the imaging plane using standard, commercially available fluorescent probes. We demonstrate the potential of dSTORM by resolving the distribution and clustering of presynaptic proteins as well as postsynaptic receptors and postsynaptic junctional folds.

1748-Pos Board B640**A Super-Resolution Study of the Structural Organization of the E. Coli FTSZ-Ring by ZapA and ZapB**

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The first step in E. coli cell division is the localization of the FtsZ protein at midcell, where it polymerizes into a ring-like structure called the Z-ring. FtsZ is widely conserved throughout bacteria and is a structural homolog of eukaryotic tubulin. Understanding the organization of the Z-ring will provide insight into the force generation mechanism of bacterial cell division.

In our previous work we used a super-resolution imaging method, photoactivated localization microscopy (PALM), to characterize the arrangement of FtsZ protofilaments inside the Z-ring¹. We showed that the Z-ring is likely composed of a loose association of protofilaments overlapping in both the circumferential and radial direction of the Z-ring. Given that FtsZ protofilaments exhibit a low intrinsic self-interaction, how they are associated in vivo remains unknown.

Recently a family of Z-ring associated proteins (Zap) have been shown to promote FtsZ polymerization in vitro and Z-ring formation in vivo. In this study, we investigated how ZapA and ZapB affect the organization of the Z-ring.

We used live-cell PALM imaging to characterize the structure of the Z-ring in the absence of ZapA and ZapB. We found that in the absence of ZapA or ZapB, FtsZ adopts a variety of non-native structures characterized by a fractured appearance containing dispersed clusters of FtsZ. Similar FtsZ clusters have been observed to precede wild-type Z-ring assembly and are likely composed of

multiple FtsZ protofilaments. Comparison of the FtsZ clusters observed in ZapA and ZapB to those observed in wild-type suggest that ZapA and ZapB may function to promote Z-ring assembly by corralling and consolidating higher-ordered FtsZ groupings.

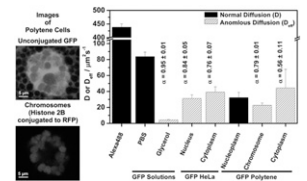
[1] G. Fu, T. Huang, J. Buss, C. Coltharp, Z. Hensel, J. Xiao, PLoS One 2010, 5, e12680.

1749-Pos Board B641**Quantifying the Contribution of Obstructions to Anomalous Diffusion in Cell Nuclei**

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Most proteins exhibit anomalous subdiffusion in cell nuclei. The source of this behavior is not well understood but is likely due to a combination of crowding and binding of proteins. In order to separate the contributions of these two effects, we measured the diffusion of unconjugated GFP in HeLa cells and the polytene cells of *Drosophila* salivary glands using two-photon excited fluorescence recovery after photobleaching (FRAP). Our experiments use a diffraction-limited bleaching and observation volume to quantitatively characterize diffusion in specific nuclear substructures. In polytene cells unlike HeLa cells, we can resolve GFP diffusion behavior in nuclear regions containing chromosomes from regions devoid of chromosomes (Figure). Interestingly, we observed anomalous diffusion of GFP in the chromosomal regions only; GFP diffuses normally in the inter-chromosomal space of the polytene nuclei (Figure). This observation indicates that obstructed diffusion through chromatin is a primary source of anomalous diffusion in cell nuclei. Our presentation will focus on technical challenges of applying quantitative point FRAP, including the need to account for reversible fluorophore photobleaching in data analysis, as well as results and interpretation of anomalous diffusion measurements of unconjugated GFP in cells.

**1750-Pos Board B642****Optical Measurement of Biomechanical Properties of Human Red Blood Cell using Digital Holographic Microscopy: Malaria and Sickle Cell Diseases**

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Pathophysiological aspect of several hematologic diseases is largely determined by biomechanical properties of red blood cells (RBCs) and their hemodynamical properties in circulatory system 1. Here we present the biomechanical properties of individual RBCs from patients with sickle cell disease and RBCs infected with malaria-inducing parasites *Pf. falciparum*. using laser digital holographic technique, we non-invasively quantify membrane fluctuation in RBCs at the nanometer and millisecond scale, which is analyzed with the mathematical model to retrieve four important mechanical properties of RBCs; bending modulus, shear modulus, area expansion modulus, and cytoplasmic viscosity 2. We find significant alterations in the mechanical properties of RBCs in several pathophysiological states, ranging from depletion of Adenosine-5'-triphosphate (ATP) 3, different osmotic pressures 4, malaria infections 5-7, and sickle cell diseases 8,9.

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1751-Pos Board B643**High Resolution Imaging of Living Cells with Microsecond Force Spectroscopy**

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